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EVALUATION OF THE EFFICACY OF THE BIOLOGICAL COMPOUND AFFECTED BY *TALAROMYCES FLAVUS* IN CONTROLLING TOMATO FUSARIUM WILT DISEASE IN THE FIELD CONDITIONS*

SHIMA FARHANG NIYA[1], LALEH NARAGHI[2], FARKHONDEH OMMATI[3] & MAHDI PIRNIA[4]

[1]M.Sc. Student of Plant Pathology, Islamic Azad University, Damghan Branch, Damghan, Iran

[2]Assistant Professor of Plant Pathology, Plant Diseases Research Department, Iranian Research Institute of Plant Protection, No.1, Yaman Street, Chamran Free Way, Tehran, Iran

[3]Researcher of Plant Pathology, Plant Protection Department, Shahrood Agricultural Research Center, Shahrood, Iran

[4]Assistant Professor of Plant Pathology, Islamic Azad University, Damghan Branch, Damghan, Iran

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ABSTRACT

Fusarium wilt is one of the most important tomato diseases. Since the pathogenic agent of this disease is soil-borne, therefore for its management, chemical control methods are not possible easily. In this regard, biological control by antagonistic soil-borne fungi is effective. At present study, based on the laboratory and greenhouse results related to previous experiments, the efficacy of four treatments affected by *Talaromyces flavus* with the most effective in decreasing disease incidence was investigated in the field conditions. These treatments included of *T. flavus* isolate No. TF-To-V-29 as addition to soil, *T. flavus* isolate No.TF-To-V-29 as both addition to soil and seed coating, *T. flavus* isolate No. TF-To-U-38 as addition to soil and *T. flavus* isolate as both addition to soil and seed coating. For preparation of *T. flavus* inoculum, every isolate of *T. flavus* was cultured on a medium substrate containing rice bran and peat moss. Field experiment was performed in a randomized complete block design with five treatments and four replications. Evaluation of the treatments were carried out as calculating disease severity percent one month after planting and continued to three months monthly. In the end of vegetative phase, the total yield was calculated for every treatment. Data were analyzed by ANOVA (Analysis of Variance) using the MS TAT C statistical software, while means were separated by the Dunkan's multiple range test. Results showed that the most effective treatment in decreasing disease severity and increasing total yield belonged to the *T. flavus* isolate No. TF-To-V-29 as both addition to soil and seed coating.

KEYWORDS: Biological Control, *Fusarium oxysporum* f. sp. *lycopersici*, *Talaromyces flavus*, Tomato

INTRODUCTION

Tomato and its conversion products are of the most important conversion industries in the world. The under cultivation area of this crop is 4 million square meters in the world and its production is 130 million tons. So that approximately 35-30 million tons of fresh tomatoes are processed in factories and the tomato paste is their main product. A total of 37 countries in the world have the possibility of processing tomatoes in which 12 countries do processing up to 90 percent. Iran in 2008, in terms of processing was placed in the fifth place after the USA, Italy, China and Turkey.

Also, Iran with an annual production of 5 million and 800 thousand tons of tomatoes, has had the seventh rank in tomato production and the tenth rank in export of tomato paste in the world (Rezvani Moghaddam *et al*, 2011). It can be said in terms of the importance of this plant that:

- According to the latest statistics, the average yields obtained from a hectare of cultivated tomato is 37 tons that this was 40 and 53 tons in Khorasan Razavi and Hamadan province, respectively (Jadidi *et al*, 2012).
- Nowadays, a great industry is responsible for tomato processing and provides it to customers in forms of dried, peeled, puree, paste, various kinds of sauce such as ketchup, powder and juice. Condensation and drying are two main types of industrial tomato processing.
- Tomato is rich in vitamins needed by the human body that always should be included in household food basket.
- Each hectare of tomato cultivation creates 211 jobs for people per day of production season.
- Tomato is of crops which has the highest water use efficiency in agriculture.

The Fusarium wilt is a fungal disease which is caused by *Fusarium oxysporum* f. sp. *lycopersici*. The above-mentioned fungus is a soil-borne and is spread throughout the world. The pathogen enters the plant through the root and affects the plant vascular system usually in seedling stage. The plants which are infected in the growth stage will be destroyed. The symptoms are more evident in warm areas (Jones *et al*, 1991).

The Fusarium wilt disease can attack the plant in different stages of growth and the first symptoms are as yellowing and wilting of the leaves of one side of the plant or a branch or sometimes in some branches. Yellowing and wilting of the plant spread with the expansion of fungus. Also, the premature loss of leaves, browning of the vascular system, poor fruits, no yielding of fruits, weakening of plants and plant death, all occur in plants before reaching the age of puberty (Bravo-Ruiz *et al*, 2013).

The vascular wilt are of most important symptoms which emerges as discolor veins in the young leaves, wilting of lower older leaves and finally the death plant. Sometimes, the species resistant to Fusarium wilt show mild symptoms of the disease. In particular, this occurs when some wounds are caused by nematodes or the plant has been damaged in the root by asphyxia (Fravel *et al*, 2003).

So far, the most common way to control this disease has been related to the use of resistant varieties with fielding practices. Among other methods, can be mentioned the biological inhibitor factors which in some cases, in addition to disease control, enhance the growth of plants as well (Etebarian *et al*, 2000; Niknejad Kazempour *et al*, 2000; Hajieghrari *et al*, 2005).

According to previous research, when choosing a microorganism as a biological control agent, must ensure that natural conditions dominant on the environment don't have adverse effects on reproduction and activity of the control agent in the intended area (Renwick and Jones, 1986; Nelson, 1988). Another important reason for the lack of a biological control agent, is using it in an inappropriate way (Myung, 2008).The research was conducted as a field experiment and followed by previous research laboratory and greenhouse studies (Atfannejad Dezfooli *et al*, 2014)

According to the laboratory studies conducted on 24 isolates of Antagonistic fungi (*Talaromyces flavus*), three effective isolates of each fungus had been selected for the greenhouse experiment. Also, in greenhouse experiments with

the main treatments (various methods used for antagonist isolates) and secondary treatments (effective isolates selected through the laboratory studies), finally, the top four treatments affected by antagonistic isolates were selected with their application methods for field studies. The purpose of this study was to determine the effectiveness of the introduced treatments in the control of Fusarium wilt of tomato in the field conditions.

MATERIALS AND METHODS

Choosing the Field Treatments

According to previous studies (Atfannejad Dezfooli *et al*, 2014) among the 9 isolates of *Trichoderma harzianum* and 24 isolates isolate of *Talaromyces flavus*, three isolates were selected for the greenhouse experiment from each fungi with the highest inhibition percentage for pathogen growth in *in vitro*. In addition, in a greenhouse experiment with main treatments (various methods used for antagonistic isolates) and secondary treatment (effective isolates selected through the laboratory studies), finally, the top four treatments affected by antagonistic isolates were selected with their application methods for field studies. Finally, in this study, were determined the effects of top treatments on of disease severity percent of Fusarium wilt of tomato.

These treatments given the isolates and application methods, were as follows: 1- TF-To-V-24 by the method of adding to the soil and impregnating the seed, 2- TF-To-V-29 by the method of adding to the soil and impregnating the seed, 3- TF-To-U-38 by the method of adding to the soil and impregnating the seed,4- TF-To-U-38 by the method of adding to the soil. It should be noted that all of these isolates were of *Talaromyces flavus* isolates which were obtained from the soil of tomato fields in Varamin (isolate TF-To-V-24 and TF-To-V-29) and Urmia (TF-To-U-38).

Providing the Inoculum Affected by the *T. flavus* Isolates

To provide the used inoculum through the relevant isolates, was used the method changed by Naraghi *et al*. (2010). So that an amount of rice bran was soaked for 24 hours in water at temperature (35-30^{0}C). Then they were spread on large filter papers and dried. Next, 200 grams of rice bran and 50 g of washed peat soil were sterilized by cellophane bags in autoclave (pressure of 1 atmosphere, temperature of 120 ° C for 15 min). Then, for supplying the inoculum needed for each isolate, a suspension containing 20 ml of sterile distilled water and four one-cm pieces of a 10-day cultured environment of the related isolate, were placed in cellophane bags.

For the growth of the isolates, the cellophane bags were placed in the 30^{0}C incubator for a month and a half to two months and during this period, if drying the contents, again, to create humidity, was added 20 ml of sterile distilled water. After this period, the contents in each cellophane bag were spread on filter papers for drying and were used as the consumed inoculum on the field in two forms of adding to the soil and impregnating the seeds.

Field Study

The study was conducted in a field located in the Shahrood Research Station with a history of infection with Fusarium wilt. In each crop year, the experiment was conducted in completely randomized blocks with five treatments (four treatments with the greatest impact on reducing disease in greenhouse conditions and one control treatment) and four replications. For each replication, a plot including four 6-meter rows and between every two plots, a row was located without treatment or the main plots. In this study was used the Mobil cultivar (the common tomato cultivar for cultivation in the region).

Since the application of treatment was affected by the antagonistic isolates through the method of adding to the soil, based on the use, 50 kg biological fungi inoculum per ha with CFU /g 109 or Colony Forming Unit (CFU) was applied for its every grams (Ruppel *et al*, 1983; Van Toor *et al*, 2002). So that after calculating the number of spores of the Antagonistic isolate per each gram of relevant inoculum by lam haemocytometer and blanking it to achieve the number of CFU /g 10E9 through the re-cultivation of Antagonistic on bed of the inoculum (if was lower than the mentioned number) or the addition of the substrate free of the Antagonistic isolate to the inoculum (if was higher than the mentioned number) was determined the used amount for the experiment area. For example, for a treatment with four replications with an area of 500 square meters, 2.5 kg inoculum was used. For treatments that the application of the Antagonistic isolate is as impregnation of the seeds, as the use of these treatments in the greenhouse, the inoculum is used until that whole of seed's surface be impregnated with it (Naraghi *et al*, 2010).

To ensure the necessary pollution needed for the field, namely a minimum area of 0.9×10^3 to 1.345×10^3 or the Colony Forming Unit (CFU) per gram of soil, the test of pathogenic fungi population was conducted through soil cultivation on specific Komada's Medium according to Komada method (1975). If the Colony Forming Units of the pathogenic fungi per gram of soil field was lower than the above number, before conducting the experiment, a synthetic inoculum was prepared according to the method, on a bed of sand and corn with the ratio of 1: 20 and the amount if the needed inoculum with a concentration of CFU/g 10E3 (Leeman *et al*, 1997) was distributed throughout the soil field on the basis of 33 kg per hectare (Elmer, 2002) and was quite mixed with it to a depth of 15 cm (Hashimoto *et al*, 2008).

Disease Assessment

Evaluation of Fusarium wilt disease as determining the disease indicator according to Hao *et al*. (2005), started a month after planting and continued for four months (Wokoma, 2008). Finally, a comparison between treatments was done by analyzing data through the Duncan's Multiple Range test and using the software MS TAT C.

According to method introduced by Hao *et al*. (2005), first, the incidence of the disease was determined using a scale of six degrees (Liu et al, 1995) as follows:

0= no symptoms

1= leaf chlorosis and plant wilting less than 25%

2= leaf chlorosis and plant wilting from 25 to 50%

3= leaf chlorosis and plant wilting from 51 to 75%

4= leaf chlorosis and plant wilting from 76 to 100%

5= Plant is dead or completely missed

Then, the disease severity percent for each treatment was calculated according to the following formula:

$\frac{\text{The number of plants with degree No.0+ The number of plants with degree No.1+ The number of plants with degree No.2+ The number of plants with degree No.3+ The number of plants with degree No.4+ The number of plants with degree No.5}}{\text{The total number of plants}} \times 100$	=percentage of disease severity

RESULTS

Comparison of Disease Severity Percent in the Treatments

The experiment of the effect of the treatments affected by *T. flavus* on the severity of the Fusarium wilt of tomato was significant at 5% level. During the statistical classification of disease severity percentage means, the experiment treatments were placed in three statistical groups. The lowest mean percentage of disease severity percent (33.92%) was observed in the treatment affected by the isolate TF-To-V-29 with method of adding to the soil. In addition, the highest mean percentage of disease severity percent (42.50%) was belonged to the control treatment (Table 1).

On the other hand, a reduction in the percentage of the disease severity percent was observed in all treatments affected by antagonistic isolates compared to the control and there was no statistically significant difference among other treatments (the isolate TF-To-V-29 as addition to the soil and seed coating, the isolate, TF-To-U-38 as addition to the soil and the isolate TF-To-U-38 as addition to the soil and seed coating) (Table 1).

Table 1: Comparison of Mean Percentages of Disease Severity Percent of the Fusarium Wilt Disease in the Treatments Affected by *Talaromyces flavus* in the Tomato Field of Sharood in Year 2013

Treatment	The Mean of Disease (%) Severity Percent
TF-To-V-29 as addition to the soil	33.92* a
TF-To-V-29 as addition to the soil and seed coating	35.62ab
TF-To-U-38 as addition to the soil	36.75ab
TF-To-U-38 as addition to the soil and seed coating	34.50ab
Control	**42.50a**

*: There is no significant difference at the level of 5% between similar statistical letters.

Comparison of the First Harvesting Yield in the Treatments

The experiment of the effect of the treatments affected by *T. flavus* on the tomato yield in the first harvesting was not significant at 5% level. Thus, there was no significant difference between treatments, but in the treatments affected by the isolate TF-To-U-38 as addition to the soil, compared to the control, a 12.97 percent of increased yield existed in the first harvesting (Table 2).

Table 2: Comparison of the Yield Means Related to the First Harvesting in the Treatments Affected by *Talaromyces flavus* in the Tomato Field of Sharood in Year 2013

Treatment	First Harvesting (Kg/ ha) Mean
TF-To-V-29 as addition to the soil	2265.83* a
TF-To-V-29 as addition to the soil and seed coating	4651.25a
TF-To-U-38 as addition to the soil	3598.83 a
TF-To-U-38 as addition to the soil and seed coating	4425.42a
Control	**3185.42a**

*: There is no significant difference at the level of 5% between similar statistical letters.

Comparison of the Second Harvesting Yield in the Treatments

The experiment of the effect of the treatments affected by *T. flavus* isolates on the tomato yield in the second harvesting was significant at 5% level. Due to the statistical grouping of yield means in the second harvesting, the test

treatments were placed in three statistical groups. Among all treatments, the highest average yield of the second cutting (11009.58 kg per hectare) was belonged to the treatment affected by the isolate TF-To-V-29 as addition to the soil and seed coating. Also, the highest yield mean in the second harvesting (7355.83 kg per hectare) was observed in the isolate TF-To-V-29 as addition to the soil. There was no significant difference between other treatments in terms of yield mean in the second harvesting (Table 3)

Table 3: Comparison of the Yield Means Related to the Second Harvesting in the Treatments Affected by *Talaromyces flavus* in the Tomato Field of Sharood in Year 2013

Treatment	Second Harvesting Mean (Kg/ ha)
TF-To-V-29 as addition to the soil	7355.83b*
TF-To-V-29 as addition to the soil and seed coating	11009.58a
TF-To-U-38 as addition to the soil	9385.00 ab
TF-To-U-38 as addition to the soil and seed coating	8454.84ab
Control	**8391.25ab**

*: There is no significant difference at the level of 5% between similar statistical letters.

Comparison of the Third Harvesting Yield in the Treatments

The experiment of the effect of the treatments affected by T. flavus on the tomato yield in the third harvesting was significant at 5% level. Due to the statistical grouping of yield means in the third harvesting, the treatments were placed in four statistical groups. Among all treatments, the highest yield mean belonged to the treatment affected by the isolate TF-To-V-29 as addition to the soil and seed coating (17983.33 kg per hectare) and the isolate TF-To-V-38 as addition to the soil (13674.99 kg per hectare). While there was no significant difference between other two treatments (the isolate TF-To-V-29 as addition to the soil and the isolate TF-To-V-38 as addition to the soil and seed coating) in terms of yield mean in the third harvesting (Table 4).

Table 4: Comparison of the Yield Means Related to the Third Harvesting in the Treatments Affected by *Talaromyces Flavus* in the Tomato Field of Sharood in Year 2013

Treatment	(Kg/ ha) Third Harvesting Mean
TF-To-V-29 as addition to the soil	9929.16b*
TF-To-V-29 as addition to the soil and seed coating	17983.33a
TF-To-U-38 as addition to the soil	13679.94ab
TF-To-U-38 as addition to the soil and seed coating	10891.66b
Control	**11899.99b**

*: There is no significant difference at the level of 5% between similar statistical letters.

Comparison of the Forth Harvesting Yield in the Treatments

The experiment of the effect of the treatments affected by *T. flavus* on the tomato yield in the forth harvesting was significant at 1% level. Due to the statistical grouping of the yield means in the forth harvesting, the treatments were placed in four statistical groups. Among all treatments, the highest yield mean belonged to the treatment affected by the isolate TF-To-V-29 as addition to the soil and seed coating (16020.83 kg per hectare) and the isolate TF-To-U-38 as addition to the soil (13633.33 kg per hectare). The lowest yield mean in the forth harvesting was observed in the treatment affected by the isolate TF-To-V-38 as addition to the soil and seed coating. On the other hand, there was no significant difference between the control (12141.74 kg per hectare) and the isolate TF-To-V-29 as addition to the soil (10954.16 kg per hectare) (Table 5).

Table 5: Comparison of the Yield Means Related to the Forth Harvesting in the Treatments Affected by *Talaromyces flavus* in the Tomato Field of Sharood in Year 2013

Treatment	Forth Harvesting (Kg/ ha) Mean
TF-To-V-29 as addition to the soil	10954.16 bc*
TF-To-V-29 as addition to the soil and seed coating	16020.83a
TF-To-U-38 as addition to the soil	13633.33a
TF-To-U-38 as addition to the soil and seed coating	8858.33a
Control	**12141.74bc**

*: There is no significant difference at the level of 1% between similar statistical letters.

Comparison of the Fifth Harvesting Yield in the Treatments

The experiment of the effect of the treatments affected by *T. flavus* on tomato yield in the fifth harvesting was significant at 5% level. Due to the statistical grouping of the yield means in the fifth harvesting, the treatments were placed in three statistical groups. Among all treatments, the highest yield mean belonged to the treatment affected by the isolate TF-To-V-29 as addition to the soil and seed coating (11370.83 kg per hectare). Howevere, the lowest average yield of the fifth cutting was observed in the treatment affected by the isolate TF-To-V-29 as addition to the soil (7162.49 kg per hectare). On the other hand, there was no significant difference between the control (6937.49 kg per hectare) and the isolate TF-To-V-38 as additionto the soil (8441.66 kg per hectare) and the isolate TF-To-V-38 as addition to the soil and seed coating (6937.49 kg per hectare) (Table 6).

Table 6: Comparison of the Yield Means Related to the Fifth Harvesting in the Treatments Affected by *Talaromyces flavus* in the Tomato Field of Sharood in Year 2013

Treatment	(Kg/ ha) Fifth Harvesting Mean
TF-To-V-29 as addition to the soil	7169.42b*
TF-To-V-29 as addition to the soil and seed coating	11370.83 a
TF-To-U-38 as addition to the soil	8441.66 ab
TF-To-U-38 as addition to the soil and seed coating	8995.83ab
Control	**6637.49ab**

*: There is no significant difference at the level of 5% between similar statistical letters.

Comparison of the Yield Means Related to the Sixth Harvesting in the Treatments

The experiment of the effect of the treatments affected by *T. flavus* on the tomato yield in the sixth harvesting was not significant at 5% level. However, in treatments affected by the isolate TF-To-V-29 as addition to the soil and seed coating and the isolate TF-To-U-38 as addition to the soil compared to the control, there were 24.03 and 13.95 percent increase in the yield mean, respectively (Table 7).

Table 7: Comparison of the Sixth Harvesting Yield in the Treatments Affected by *Talaromyces flavus* in the Tomato Field of Sharood in Year 2013

Treatment	Fifth Harvesting Mean (Kg/ ha)
TF-To-V-29 as addition to the soil	2520.83 a*
TF-To-V-29 as addition to the soil and seed coating	3333.33 a
TF-To-U-38 as addition to the soil	3062.49a
TF-To-U-38 as addition to the soil and seed coating	2979.16a
Control	**2687.44 a**

*: There is no significant difference at the level of 5%, between similar statistical letters.

Comparison of Total Yield in the Treatments

The experiment of the effect of the treatments affected by *T. flavus* on the tomato total yield was significant at 5% level. Due to the statistical grouping of the yield means, the treatments were placed in five statistical groups. Based on the results of this section, in all treatments affected by *T. flavus* isolates, comparing to the control observed a significant increase in total yield. Among these treatments, the highest yield mean belonged to the treatment affected by the isolate TF-To-V-29 as addition to the soil and seed coating (68244.14 kg per hectare). However, the lowest total yield mean was observed in the treatment affected by the isolate TF-To-V-29 as addition to the soil (40588.49 kg per hectare) (Table 8).

Table 8: Comparison of the Total Yield Means in the Treatments Affected by *Talaromyces flavus* in the Tomato Field of Sharood in Year 2013

Treatment	(Kg/ ha) Fifth Harvesting Mean
TF-To-V-29 as addition to the soil	40588.31c*
TF-To-V-29 as addition to the soil and seed coating	48244.14a
TF-To-U-38 as addition to the soil	49321.22 b
TF-To-U-38 as addition to the soil and seed coating	45171.22 bc
Control	**44643.31d**

*: There is no significant difference at the level of 5% between similar statistical letters.

DISCUSSIONS AND CONCLUSIONS

The total results of this study showed that there is a possibility to biologically struggle with Fusarium wilt disease derived from *F. oxysporum* in the tomato crop by some isolates of *T. flavus*. Isolation of various *T. flavus* isolates from tomato growing areas shows that this fungi was naturally existed in the rhizosphere of tomato (Papavizas, 1995). *T. flavus* occupies the rhizosphere of the crop and reducing the colony forming units of the Fusarium wilt pathogenic agents (Marois *et al*, 1984; Whipps, 2001; Tsahouridou and Thunassoulopolos, 2002; Roberts and Lohrke, 2003; Tjamos *et al*, 2004; Berg *et al*, 2005).

In this study, the lowest Disease severity percent has been related to the isolate TF-To-V-29 as addition to the soil. The results of a research in the field of biological control of *Sclerotinia sclerotiorumin* in fields of beans and peas by the antagonistic fungus *Tricothecium roseum*, *Trichoderma virens*, *Coniothyrium minitans* and *T. flavus* showed that the efficacy of the mentioned antagonistic fungus in the treatment of addition to the soil, was more due to the increase in establishment and their sustainability (Chang and Scott, 2000). In comparative studies between different methods of the application of the treatments affected by antagonistic microorganisms, increase in the efficiency of these microorganisms was significantly higher by the method of addition to the soil (Josh and Pan, 2007; Joshi *et al*, 2010; Patkowska and Konopinski, 2014).

In this study, the treatment affected by isolate TF-To-V-29 when increase in soil, comparing with its application with the method of adding to the soil and seed coating, was superior in terms of the effectiveness of disease control. Based on this results, it can be inferred that the population of the spores of the isolate TF-To-V-29 in the method of adding to the soil was only to an extent that no barrier was created to the antagonistic activity of this isolates; but about the use of this isolate, with the method of adding to the soil and seed coating, perhaps the increase in fungal population reduces the antagonistic activity. Previous studies have shown that the use of high concentrations of antagonistic fungus such as materials Aspergillus and Penicillium led to emergence of a phenomena entitled "Crowding effect". In this phenomenon, high population of fungal spores led to production of inhibitor materials for the growth of spores.

For example, population of fungal spores of *Penicillium paneum*, due to producing the 1- octen-3-OL compound (a mixture resulted from linoleic acid oxidation) with four mM concentration, will prevent the growth of spores, so that the activity of antagonistic fungi decreases. Therefore, in the present research, it has been likely that the propagator bed of the antagonist isolate acts as an incentive to increase the population of antagonistic isolates' spores and due to the mentioned phenomena, could not effect on the increase in the antagonism activity of isolates (Chitarra, 2003).

On the other hand, in this study, the reason of the superiority of isolate TF-To-V-29 on TF-To-U-38, in terms of the effectiveness of disease control, can be attributed to genetic differences and differences in their isolation areas. The results of research on the genetic diversity of different isolates of the antagonistic fungi have shown that the different intensity of enzymatic activity of the isolates was caused by genetic variation (Choudary *et al*, 2007; Siameto *et al*, 2010). In addition, the accumulation of the effective metabolites with inhibitory properties for the growth of pathogens in these isolates, was related to the favorable environmental conditions for antagonist's growth and activity (Joshi *et al*, 2010).

In this study, in terms of yield, there was no statistically significant difference between treatments in the first cutting and the significant difference started in the second cutting and continued to the fifth cutting. Therefore, based on these findings, the difference in the activity of antagonist treatments in terms of increase in performance, is exactly compatible to the stages of plant growth that there was a physiological readiness for crop production. In the six cutting, treatments were placed in one group in terms of average performance. It seems that in this step, the activity of the antagonistic isolate reduced due to the decrease in their population and new inoculation is necessary for next crop year, as well. In some studies on the biological control of soil-borne diseases of potato such as Verticillium wilt by using antagonistic fungi, the modification of these soil microorganisms in fields has been reported through applying the treatments affected by Antagonistic fungi (Lodhi, 2004; Berg *et al*, 2005; Naraghi *et al*, 2014).

Finally it is hoped that the results of this study can be used for biological control of Fusarium wilt disease of tomato and plays a minor role to achieve the goals of sustainable agriculture which are increase in agricultural production, reduce in the use of chemical pesticides and protection of the environment and biological resources.

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